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## (54) Title: GENES ENCODING SULFATE ASSIMILATION PROTEINS

## (57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a sulfate assimilation protein. The invention also relates to the construction of a chimeric gene encoding all or a portion of the sulfate assimilation protein, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the sulfate assimilation protein in a transformed host cell.

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1
SEQ ID NO:2 -----
MTA-GQLLRTEPSAQ-----PQRVR-HSTPPAALQADIVFSY
SEQ ID NO:4 -----MP-VQEQLQKTSVPAQDQVENVVE-----QPRR-HS--PPALHPAVVFAY
SEQ ID NO:6 -----MTA-GQFLRLDDP-----A-NSL
SEQ ID NO:8 -----
SEQ ID NO:10 -----
SEQ ID NO:12 -----MP---TGL----PA-----ARA
SEQ ID NO:14 -----
SEQ ID NO:16 -----MTA-GQPLRLRADP-----QORR-HS--PPALHPAVVFAY
SEQ ID NO:17 -----MP--VGELRFSSQS-----S---TTV
SEQ ID NO:18 -----MPP-AGELRHQSPSKEKLSSVTQSDEAEA-----AS---AAI
SEQ ID NO:19 -----MAACIDTCRTGKPQISPROSSKHHDESGFRYMFYRYPDRSSFNGTQTTLHTR--PLL

61
SEQ ID NO:2 -----
PPPESGDDESWVWSQIKAEARRDADAEPALASFLYATVLSHASLDRSLAFOLANKLCSST
SEQ ID NO:4 -----DAEESGVWSQIKAEARRDAESEPALASLYSTILSHSSLAASLSFHGNKLCSS
SEQ ID NO:6 -----PPPESDADESWVWSQIKAEARRDADAEPALASFLYATVLSHPSLDRSLAFHLANKLCSST
SEQ ID NO:8 -----
SEQ ID NO:10 -----
SEQ ID NO:12 -----
SEQ ID NO:14 -----
SEQ ID NO:16 -----
SEQ ID NO:17 -----
SEQ ID NO:18 -----
SEQ ID NO:19 -----SAAAADAEEAGLWTQIKAEARRDADAESEPALASLYSTILSHSSLERSISFHGNKLCSS
EDLDROAEVDDVWAKIREEAKSDIAKEPIVSAYHASICVSQRSLEAALANTLSVKLSNLN

121
SEQ ID NO:2 -----
LLSTLLYELFVASLAEPHYVRAAAVADLIARRSRPG-----LNYKGFLAIQAHRVA
SEQ ID NO:4 -----LLSTLLYDLFLGVLSLSDSLRUAAVADLRAARQRPACTSFHCLLNKYKGLIAQARV
SEQ ID NO:6 -----LLSTLLYDLFVASLAAPHTLRAAVVADLRAARSRDPAVCVGFSCHLNLYKGLIAQARV
SEQ ID NO:8 -----LLSTLLYDLFLASFTAPHSRLRAAVVADLRAARSRDPAVCVGFSCQCLLNFKGLIAQARV
SEQ ID NO:10 -----LLSTLLYDLFLNFAFSDPSLRSAAVADLRAARERDPACVSYSHCLLNKYKGLACOAHRVA
SEQ ID NO:12 -----
SEQ ID NO:14 -----
SEQ ID NO:16 -----
SEQ ID NO:17 -----
SEQ ID NO:18 -----
SEQ ID NO:19 -----LLSTLLYDLFVGSLAAHPTIRAAA VADLLAVRSR-----LNYKGFLAIQAHRVA
LLSTLLYDLFLNFSSDPSLRLNATVADLRAARVDPACISFSHCLLNKYKGLACOAHRVA
LLSTLLYDLFLNFTSDPSLRLNATVADLRAARVDPACISFSHCLLNKYKGLACOAHRVA
LPSNTLDFLSGVLGQNPDIVESVKLDDLAVKERDPACISYVHCFLHFKGFLACOAHRIA

181
SEQ ID NO:2 -----HVLWAQQRRPLALALQSRVADVFADVFADHPIAAVVGKGIILDHATGVVIGETAVVGDNVSL
SEQ ID NO:4 -----
SEQ ID NO:6 -----HKKWMSQRKPLSLALQSRIDAVFSDVPHIAPARIGKGVLLDHATGVVIGETAVIGNVSL
SEQ ID NO:8 -----HVLWAQDRRRALALQSRVAEVFADFIDHPPAAIGKGVLLDHATGVVIGETAVIGNVSL
SEQ ID NO:10 -----HVLWAQQRRLPLALQSRVADEFADFIDHPPAAVVGKGIILDHATGVVIGETAVVGDNVSL
SEQ ID NO:12 -----HLLWRQSRRLPLALHSRIDADEFADFIDHPPPARIGKGVLLDHATGVVIGETAVVGDNVSL
SEQ ID NO:14 -----HKLWLQGRKVLLALIQNRVSEEVADFIDHPPGAKIGRGLLHDHATGLVVGETAVIGNVSL
SEQ ID NO:16 -----
SEQ ID NO:17 -----HKLWNOSRRPLALALQSRIDADEFADFIDHPPAARIGKGVLLDHATGVVIGETAVIGNVSL
SEQ ID NO:18 -----
SEQ ID NO:19 -----HELWTQDRKILALLIQNRVSEAFADFHPGAKIGTGTGILLDHATAIVIGETAVVGNVSL

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TITLE

## GENES ENCODING SULFATE ASSIMILATION PROTEINS

This application claims the benefit of U.S. Provisional Application No. 60/092,833, filed July 14, 1998.

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FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding sulfate assimilation proteins in plants and seeds.

BACKGROUND OF THE INVENTION

10       Sulfate assimilation is the process by which environmental sulfur is fixed into organic sulfur for use in cellular metabolism. The two major end products of this process are the essential amino acids cysteine and methionine. These amino acids are limiting in food and feed; they cannot be synthesized by animals and thus must be acquired from plant sources. Increasing the level of these amino acids in feed products is thus of major  
15       economic value. Key to that process is increasing the level of organic sulfur available for cysteine and methionine biosynthesis.

Multiple enzymes are involved in sulfur assimilation. These include: High affinity sulfate transporter and low affinity sulfate transporter proteins which serve to transport sulfur from the outside environment across the cell membrane into the cell (Smith et al.  
20       (1995) *PNAS* 92(20):9373-9377). Once sulfur is in the cell sulfate adenylyltransferase (ATP sulfurylase) (Bolchia et al. (1999) *Plant Mol. Biol.* 39(3):527-537) catalyzes the first step in assimilation, converting the inorganic sulfur into an organic form, adenosine-5' phospho-sulfate (APS). Next several enzymes further modify organic sulfur for use in the biosynthesis of cysteine and methionine. For example, adenylylsulfate kinase (APS kinase),  
25       catalyzes the conversion of APS to the biosynthetic intermediate PAPS (3'-phospho-adenosine - 5' phosphosulfate) (Arz et al. (1994) *Biochim. Biophys. Acta* 1218(3):447-452). APS reductase (5' adenylyl phosphosulphate reductase) is utilized in an alternative pathway, resulting in an inorganic but cellularly bound (bound to a carrier), form of sulfur (sulfite) (Setya et al. (1996) *PNAS* 93(23):13383-13388). Sulfite reductase further reduces the  
30       sulfite, still attached to the carrier, to sulfide and serine O-acetyltransferase converts serine to O-acetylserine, which will serve as the backbone to which the sulfide will be transferred to from the carrier to form cysteine (Yonetura-Sakakibara et al. (1998) *J. Biolchem.* 124(3):615-621 and Saito et al. (1995) *J. Biol. Chem.* 270(27):16321-16326).

As described, each of these enzymes is involved in sulfate assimilation and the  
35       pathway leading to cysteine biosynthesis, which in turn serves as an organic sulfur donor for multiple other pathways in the cell, including methionine biosynthesis. Together or singly these enzymes and the genes that encode them have utility in overcoming the sulfur limitations known to exist in crop plants. It may be possible to modulate the level of sulfur

containing compounds in the cell, including the nutritionally critical amino acids cysteine and methionine. Specifically, their overexpression using tissue specific promoters will remove the enzyme in question as a possible limiting step, thus increasing the potential flux through the pathway to the essential amino acids. This will allow the engineering of plant tissues with increases levels of these amino acids, which now often must be added a supplements to animal feed.

#### SUMMARY OF THE INVENTION

The instant invention relates to isolated nucleic acid fragments encoding sulfate assimilation proteins. Specifically, this invention concerns an isolated nucleic acid fragment 10 encoding a serine O-acetyltransferase and an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding a serine O-acetyl-transferase. In addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding serine O-acetyltransferase. An additional embodiment of the instant invention pertains to a polypeptide encoding all or a 15 substantial portion of a serine O-acetyltransferase.

In another embodiment, the instant invention relates to a chimeric gene encoding a serine O-acetyltransferase, or to a chimeric gene that comprises a nucleic acid fragment that is complementary to a nucleic acid fragment encoding a serine O-acetyltransferase, operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in 20 production of levels of the encoded protein in a transformed host cell that is altered (i.e., increased or decreased) from the level produced in an untransformed host cell.

In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding a serine O-acetyltransferase, operably linked to suitable regulatory sequences. Expression of the chimeric gene results in 25 production of altered levels of the encoded protein in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

An additional embodiment of the instant invention concerns a method of altering the 30 level of expression of a serine O-acetyltransferase in a transformed host cell comprising: a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a serine O-acetyltransferase; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the 35 chimeric gene results in production of altered levels of serine O-acetyltransferase in the transformed host cell.

An addition embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or a substantial portion of an amino acid sequence encoding a serine O-acetyltransferase.

BRIEF DESCRIPTION OF THE  
5 DRAWINGS AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figure 1 shows a comparison of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14 and 16 and the *Citrullus lanatus* and *Arabidopsis thaliana* sequences 10 (SEQ ID NO: 17, 18 (gi 2146774) and 19 (gi 1107505) respectively).

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing 15 attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1  
Sulfate Assimilation Proteins

Protein	Clone Designation	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Serine O-Acetyltransferase	Contig composed of: cc01.pk0007.h3 cen3n.pk0172.h5	1	2
Serine O-Acetyltransferase	Contig composed of: cr1n.pk0085.c5 csc1c.pk005.p2 p0022.cglnf80r p0022.cglnf80rb p0060.corac71r	3	4
Serine O-Acetyltransferase	ids.pk0030.b6	5	6
Serine O-Acetyltransferase	rlr24.pk0069.a11	7	8
Serine O-Acetyltransferase	rlr24.pk0073.d4	9	10
Serine O-Acetyltransferase	sr1.pk0162.a9	11	12
Serine O-Acetyltransferase	srm.pk0021.f11	13	14
Serine O-Acetyltransferase	wlmk4.pk0002.h5	15	16

20

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The

symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

#### DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a 5 "nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. A nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or 10 more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be 15 assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in 20 one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting 25 transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

For example, it is well known in the art that antisense suppression and co-suppression 30 of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in 35 the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for

glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed 5 modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C), with the nucleic acid fragments disclosed herein.

10 Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Preferred are those nucleic acid fragments whose nucleotide sequences encode amino acid sequences that are 90% identical to the amino acid sequences 15 reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are 95% identical to the amino acid sequences reported herein. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment 20 (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

25 A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see 30 also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods 35 of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a

nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the 5 sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

“Codon degeneracy” refers to divergence in the genetic code permitting variation of 10 the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the “codon-bias” exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. 15 Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

“Synthetic nucleic acid fragments” can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. 20 These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. “Chemically synthesized”, as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis 25 can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a 30 survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, 35 comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature.

“Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene 5 that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a nucleotide sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the 10 associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream 15 elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise 20 synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most 25 times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

30 The “translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have 35 been described (Turner and Foster (1995) *Molecular Biotechnology* 3:225).

The “3' non-coding sequences” refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The

polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into polypeptide by the cell. “cDNA” refers to a double-stranded DNA that is complementary to and derived from mRNA. “Sense” RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. “Antisense RNA” refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. “Functional RNA” refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term “operably linked” refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term “expression”, as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. “Antisense inhibition” refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. “Overexpression” refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. “Co-suppression” refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

“Altered levels” refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

“Mature” protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. “Precursor” protein refers to the primary product of translation of mRNA; i.e., with pre- and

propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*: Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

Nucleic acid fragments encoding at least a portion of a several sulfate assimilation protein have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other serine O-acetyltransferase enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific

oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673; Loh et al. (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of serine O-acetyltransferase in those cells. This enzyme is involved in sulfate assimilation and the pathway leading to cysteine biosynthesis, which in turn serves as an organic sulfur donor for multiple other pathways in the cell,

including methionine biosynthesis. This enzyme and the gene(s) that encodes the protein has utility in overcoming the sulfur limitations known to exist in crop plants. It may be possible to modulate the level of sulfur containing compounds in the cell, including the nutritionally critical amino acids cysteine and methionine. Specifically, their overexpression using tissue specific promoters will remove the enzyme in question as a possible limiting step, thus increasing the potential flux through the pathway to the essential amino acids. This will allow the engineering of plant tissues with increases levels of these amino acids, which now often must be added a supplements to animal feed.

Overexpression of the proteins of the instant invention may be accomplished by first 10 constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant 15 chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the 20 plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. 25 (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding 30 sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present 35 removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric

gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences.

Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in

5 reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant  
10 phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U. S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In  
15 addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

20 The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above,  
25 it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically  
30 measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are

microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate 5 microorganisms via transformation to provide high level expression of the encoded sulfate assimilation protein. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 6).

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part 10 of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then 15 be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted 20 and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4(1):37-41.

25 Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

30 Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

35 In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Research*

5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include 5 allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 114(2):95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nature Genetics* 7:22-28) and Happy Mapping (Dear 10 and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the 15 region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger 20 and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al. (1995) *Plant Cell* 7:75). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence 25 primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against 30 PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

#### EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention,

are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

5

### EXAMPLE 1

#### Composition of cDNA Libraries: Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, impatiens, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

10

TABLE 2

cDNA Libraries from Corn, Impatiens, Rice, Soybean and Wheat

Library	Tissue	Clone
cc01	Corn ( <i>Zea mays L.</i> ) cob of 67 day old plants grown in green house	cc01.pk0007.h3
cen3n	Corn ( <i>Zea mays L.</i> ) endosperm stage 3 (20 days after pollination)*	cen3n.pk0172.h5
cr1n	Corn ( <i>Zea mays L.</i> ) root from 7 day seedlings grown in light	cr1n.pk0085.c5
csc1c	Corn ( <i>Zea mays L.</i> ) 20 day seedling (germination under cold stress)	csc1c.pk005.p2
ids	<i>Impatiens balsamina</i> developing seed	ids.pk0030.b6
p0022	Corn ( <i>Zea mays L.</i> ) green leaves treated with jasmonic acid (1 mg/ml in 0.02% Tween 20) 24hr before collection (middle 3/4 of the 3rd leaf blade and mid rib only)***	p0022.cgiNF80r
p0060	Corn ( <i>Zea mays L.</i> ) leaf about one month after planting in green house	p0022.cgiNF80rb p0060.corac71r
rlr24	Rice ( <i>Oryza sativa L.</i> ) leaf (15 days after germination) 24 hours after infection of <i>Magaporthe grisea</i> strain 4360-R-62 (AVR2-YAMO); Resistant	rlr24.pk0069.a11
sr1	Soybean ( <i>Glycine max L.</i> ) root library	rlr24.pk0073.d4
srm	Soybean ( <i>Glycine max L.</i> ) root meristem	sr1.pk0162.a9
wlmk4	Wheat ( <i>Triticum aestivum L.</i> ) seedlings 4 hr after inoculation w/ <i>E. graminis</i> and fungicide**	srm.pk0021.f11
		wlmk4.pk0002.h5

\*This library was normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

15

\*\*Fungicide: Application of 6-iodo-2-propoxy-3-propyl-4(3H)-quinazolinone; synthesis and methods of using this compound are described in USSN 08/545,827, incorporated herein by reference.

\*\*\*Jasmonic acid is available from Sigma Chemical Co.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP<sup>\*</sup> XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP<sup>\*</sup> XR libraries are converted into plasmid 5 libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into pre-cut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid 10 vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or 15 "ESTs"; see Adams et al., (1991) *Science* 252:1651). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

#### EXAMPLE 2

##### Identification of cDNA Clones

cDNA clones encoding sulfate assimilation proteins were identified by conducting 20 BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and 25 DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX 30 algorithm (Gish and States (1993) *Nature Genetics* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the 35 cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3Characterization of cDNA Clones Encoding Serine O-Acetyltransferase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to serine O-acetyltransferase from 5 *Citrullus lanatus* (NCBI Identifier No. gi 1361979) and *Arabidopsis thaliana* (NCBI Identifier No. gi 2146774 and gi 1107505). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or contigs assembled from two or more ESTs ("Contig"):

10

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to *Citrullus lanatus* and *Arabidopsis thaliana* Serine O-Acetyltransferase

Clone	Status	BLAST pLog Score
Contig composed of: cc01.pk0007.h3 cen3n.pk0172.h5	Contig	95.70 (gi 2146774)
Contig composed of: cr1n.pk0085.c5 csc1c.pk005.p2 p0022.cglnf80r p0022.cglnf80rb p0060.corac71r	Contig	34.20 (gi 1361979)
ids.pk0030.b6	FIS	133.00 (gi 1361979)
rlr24.pk0069.a11	FIS	123.00 (gi 1361979)
rlr24.pk0073.d4	FIS	102.00 (gi 2146774)
sr1.pk0162.a9	EST	168.00 (gi 1361979)
srm.pk0021.f11	EST	69.70 (gi 1107505)
wlmk4.pk0002.h5	FIS	30.30 (gi 1361979)

Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14 and 16 and the *Citrullus lanatus* and *Arabidopsis thaliana* sequences (SEQ ID NOs:17, 18 (gi 2146774) and 19 (gi 1107505) respectively). The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14 and 16 and the *Citrullus lanatus* and *Arabidopsis thaliana* sequences (SEQ ID NOs: 17, 18 (gi 2146774) and 19 (gi 1107505) respectively).

20

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences  
of cDNA Clones Encoding Polypeptides Homologous  
to *Citrullus lanatus* and *Arabidopsis thaliana* Serine O-Acetyltransferase

SEQ ID NO.	Percent Identity to
2	82% (gi 2146774)
4	45% (gi 1361979)
6	80% (gi 1361979)
8	72% (gi 1361979)
10	81% (gi 2146774)
12	87% (gi 1361979)
14	81% (gi 1107505)
16	52% (gi 1361979)

5 Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a serine O-acetyltransferase. These sequences represent the first corn, impatiens, rice and wheat sequences encoding serine O-acetyltransferase

#### EXAMPLE 4

##### Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas,

VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight,

- 5 essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene  
10 encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al.  
15 (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum  
20 of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236)  
25 which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

- 30 The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 µm in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added  
35 to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 µL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles

resuspended in a final volume of 30  $\mu$ L of ethanol. An aliquot (5  $\mu$ L) of the DNA-coated gold particles can be placed in the center of a Kapton<sup>TM</sup> flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biostatic<sup>TM</sup> PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

#### EXAMPLE 5

##### Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the  $\beta$  subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described

above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 5 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained 10 as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with fluorescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

15 Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70, U.S. Patent No. 4,945,050). A DuPont Biostatic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a 20 chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides 25 and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µl spermidine (0.1 M), and 50 µL CaCl<sub>2</sub> (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the 30 supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an 35 empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the

retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

#### EXAMPLE 6

##### Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides

are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3)

- 5 (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio- $\beta$ -galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50  $\mu$ L of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One  $\mu$ g of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating
- 10 at the expected molecular weight.
- 15

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding a serine O-acetyltransferase comprising a member selected from the group consisting of:
  - 5 (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14 and 16;
  - (b) an isolated nucleic acid fragment that is complementary to (a).
- 10 2. The isolated nucleic acid fragment of Claim 1 wherein nucleic acid fragment is a functional RNA.
- 15 3. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13 and 15.
4. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.
5. A transformed host cell comprising the chimeric gene of Claim 4.
6. A serine O-acetyltransferase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14 and 16
- 20 7. A method of altering the level of expression of a sulfate assimilation protein in a host cell comprising:
  - 25 (a) transforming a host cell with the chimeric gene of Claim 4; and
  - (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric genewherein expression of the chimeric gene results in production of altered levels of a sulfate assimilation protein in the transformed host cell.
8. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a sulfate assimilation protein comprising:
  - 30 (a) probing a cDNA or genomic library with the nucleic acid fragment of Claim 1;
  - (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of Claim 1;
  - (c) isolating the DNA clone identified in step (b); and
  - (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)wherein the sequenced nucleic acid fragment encodes all or a substantial portion of the amino acid sequence encoding a sulfate assimilation protein.

9. A method of obtaining a nucleic acid fragment encoding a substantial portion of an amino acid sequence encoding a sulfate assimilation protein comprising:

- (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13 and 15; and
- (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a substantial portion of an amino acid sequence encoding a sulfate assimilation protein.

10. The product of the method of Claim 8.

11. The product of the method of Claim 9.

Figure 1

60

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SEQ ID NO:10	-----	-----
SEQ ID NO:12	MP--TGL----PA-----	A--NSL
SEQ ID NO:14	ARA-----	-----
SEQ ID NO:16	MTA-GOPLRADP-----	QPRR-HS--PPALHPAVVPSY
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**Figure 1 (cont'd.)**

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Val Val Ala Asp Ieu Leu Ala Ala Arg Ser Arg Asp Pro Ala Cys Val  
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Gly Phe Ser His Cys Leu Leu Asn Tyr Lys Gly Phe Leu Ala Ile Gln  
 130 135 140

Ala Gln Arg Val Ala His Val Leu Trp Ala Gln Asp Arg Arg Ala Leu  
 145 150 155 160  
 Ala Leu Ala Leu Gln Ser Arg Val Ala Glu Val Phe Ala Val Asp Ile  
 165 170 175  
 His Pro Ala Ala Ala Ile Gly Lys Gly Val Leu Leu Asp His Ala Thr  
 180 185 190  
 Gly Val Val Ile Gly Glu Thr Ala Val Ile Gly Asp Asn Val Ser Ile  
 195 200 205  
 Leu His His Val Thr Leu Gly Gly Thr Gly Lys Ala Val Gly Asp Arg  
 210 215 220  
 His Pro Lys Ile Gly Asp Gly Val Leu Ile Gly Ala Gly Ala Thr Ile  
 225 230 235 240  
 Leu Gly Asn Val Arg Ile Gly Ala Gly Ala Lys Ile Gly Ala Gly Ser  
 245 250 255  
 Leu Val Leu Ile Asp Val Pro Pro Arg Thr Thr Ala Val Gly Asn Pro  
 260 265 270  
 Ala Arg Leu Leu Gly Gly Lys Lys Gly Asp Asp Met Pro Gly Glu Ser  
 275 280 285  
 Met Asp His Thr Ser Phe Ile Gln Gln Trp Ser Asp Tyr Ser Ile  
 290 295 300

<210> 9  
<211> 1027  
<212> DNA  
<213> Oryza sativa

<210> 10  
<211> 224  
<212> PRT  
<213> *Oryza sativa*

&lt;400&gt; 10

Leu	Cys	Ser	Ser	Thr	Leu	Leu	Ser	Thr	Leu	Leu	Tyr	Asp	Leu	Phe	Leu
1				5				10					15		

Ala	Ser	Phe	Thr	Ala	His	Pro	Ser	Leu	Arg	Aia	Ala	Val	Val	Ala	Asp
			20					25					30		

Leu	Leu	Ala	Aia	Arg	Ser	Arg	Asp	Pro	Ala	Cys	Val	Gly	Phe	Ser	Gln
			35					40				45			

Cys	Leu	Leu	Asn	Phe	Lys	Gly	Phe	Leu	Ala	Ile	Gln	Ala	His	Arg	Val
			50				55				60				

Ser	His	Val	Leu	Trp	Ala	Gln	Gln	Arg	Arg	Prc	Leu	Ala	Leu	Ala	Leu
			65			70			75			80			

Gln	Ser	Arg	Val	Ala	Asp	Val	Phe	Ala	Val	Asp	Ile	His	Prc	Ala	Ala
			85					90				95			

Val	Val	Gly	Lys	Gly	Ile	Leu	Leu	Asp	His	Aia	Thr	Gly	Val	Val	Ile
			100				105				110				

Gly	Glu	Thr	Aia	Val	Val	Gly	Asp	Asn	Val	Ser	Ile	Leu	His	His	Val
			115			120					125				

Thr	Leu	Gly	Gly	Thr	Gly	Lys	Ala	Val	Gly	Asp	Arg	His	Prc	Lys	Ile
			130			135					140				

Gly	Asp	Gly	Val	Leu	Ile	Gly	Ala	Gly	Ala	Thr	Ile	Leu	Gly	Asn	Val
			145			150				155			160		

Lys	Ile	Gly	Ala	Gly	Ala	Lys	Ile	Gly	Ala	Gly	Ser	Val	Val	Leu	Ile
			165				170					175			

Asp	Val	Pro	Ala	Arg	Asn	Thr	Ala	Val	Gly	Asn	Pro	Ala	Arg	Leu	Ile
			180				185					190			

Gly	Arg	Lys	Asn	Gly	Glu	Val	Glu	Lys	Asp	Glu	Asp	Met	Prc	Gly	Glu
			195			200						205			

Ser	Met	Asp	His	Thr	Ser	Phe	Ile	Arg	Gln	Trp	Ser	Asp	Tyr	Thr	Ile
			210			215					220				

&lt;210&gt; 11

&lt;211&gt; 1131

&lt;212&gt; DNA

&lt;213&gt; Glycine max

&lt;400&gt; 11

gcacgagctg	aaccacacaa	acatcaccac	cgaacaatgc	cgacggggtt	accggcggcg	60
aattccttag	tggcgccgga	cgaagagggg	tgggtgtggg	ggcagatcaa	gcgggaggcg	120
cgccgcgacg	ccgagtcgga	gcctgccttg	gcgagctacc	tctactcgac	gtatcctctcg	180
cactcgctgc	tcaagcggttc	tctgtctttt	caccctcgaa	ataagctctg	tccctccacg	240
cttctctcga	cgccgcgttta	cgacctgttc	ctcaacgcct	tctcctccga	ccccctccctc	300
cgctccgccc	ccgtccgcga	tctccgcgt	gcccgcgaaac	gcgaccccg	ctgcgtctcc	360
tactccact	gcctcccaa	ttacaaaggc	ttccctcgct	gccaggcgca	ccgtgtggcg	420
catctgttgt	ggcgccaaatc	acggcgccca	ttggcttttg	cgctgcactc	tcgcatcgca	480
gatgtgtttg	cggtcgacat	tcaccccgccg	gcaaggattc	ggaaggggat	tttgcgtcgac	540
catgccaactg	gggtcgatgt	tagggagaca	gcgtcaatcg	ggaacaatgt	tcgcatcgatc	600
caccatgtta	ctcgccgtgg	gactggcaag	gttgggtggac	accggcatcc	taagattggg	660

gatgggggtgc ttatggtgc tggtgctacc attctgggga atattaagat tggggaaagg 720  
 gcaaagggttgc tgccggttc ggtggttta attgatgtgc caccacggac aacagcagt 780  
 gggAACCCGG CGAGGTTGGT TGGTGGGAAG GAGAAGGCCt CTAAGCATGA GGATGTGCCT 840  
 ggggagtc ta tgaccatac ttccatttac tctgagttgtt cagattataat catttgaatt 900  
 tctaaggta atcaattaaat gaatgaatac ttcaaatcaa atgctatgtt ttctgctgtt 960  
 ctagagttt gtaattttat atttggattt agattttgtt gagaccacac tctgcttaat 1020  
 tataactgttat tatgacttggaa aattttggca gcttgcataa tagtgcataa ttgtgcaca 1080  
 gattatataa agcgggtttt gttggtaaaa aaaaaaaaaaaaaaaa aaaaaaaaaaaa a 1131

<210> 12  
<211> 286  
<212> PRT  
<213> Glycine max

<400>	12		
Met Pro Thr Gly Leu Pro Ala Ala Asn Ser Leu Val Ala Pro Asp Glu			
1	5	10	15
Glu Gly Trp Val Trp Gly Gln Ile Lys Ala Glu Ala Arg Arg Asp Ala			
20	25	30	
Glu Ser Glu Pro Ala Leu Ala Ser Tyr Leu Tyr Ser Thr Ile Leu Ser			
35	40	45	
His Ser Ser Leu Glu Arg Ser Leu Ser Phe His Leu Gly Asn Lys Leu			
50	55	60	
Cys Ser Ser Thr Leu Leu Ser Thr Leu Leu Tyr Asp Leu Phe Leu Asn			
65	70	75	80
Ala Phe Ser Ser Asp Pro Ser Leu Arg Ser Ala Ala Val Ala Asp Leu			
85	90	95	
Arg Ala Ala Arg Glu Arg Asp Pro Ala Cys Val Ser Tyr Ser His Cys			
100	105	110	
Leu Leu Asn Tyr Lys Gly Phe Leu Ala Cys Gln Ala His Arg Val Ala			
115	120	125	
His Leu Leu Trp Arg Gln Ser Arg Arg Pro Leu Ala Leu Ala Leu His			
130	135	140	
Ser Arg Ile Ala Asp Val Phe Ala Val Asp Ile His Pro Pro Ala Arg			
145	150	155	160
Ile Gly Lys Gly Ile Leu Phe Asp His Ala Thr Gly Val Val Val Arg			
165	170	175	
Glu Thr Ala Ser Ile Gly Asn Asn Val Ser Ile Leu His His Val Thr			
180	185	190	
Leu Gly Gly Thr Gly Lys Val Gly Gly Asp Arg His Pro Lys Ile Gly			
195	200	205	
Asp Gly Val Leu Ile Gly Ala Gly Ala Thr Ile Leu Gly Asn Ile Lys			
210	215	220	
Ile Gly Glu Gly Ala Lys Val Gly Ala Gly Ser Val Val Leu Ile Asp			
225	230	235	240

Val Pro Pro Arg Thr Thr Ala Val Gly Asn Pro Ala Arg Lys Val Gly  
245 250 255

Gly Lys Glu Lys Pro Ser Lys His Glu Asp Val Pro Gly Glu Ser Met  
260 265 270

Asp His Thr Ser Phe Ile Ser Glu Trp Ser Asp Tyr Ile Ile  
275 280 285

<210> 13  
<211> 722  
<212> DNA  
<213> Glycine max

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<400> 13
cggcacgagc tcacaaaattg tggcttcaag ggaggaaggc cttggcgctc ttgattcaga 60
atagggtgtc tgagggtttt gctgtggata ttccaccctgg tgccaaaatt ggacgtggga 120
tttgcttggaa tcatccaaca ggacttgttgg tgggggagac tgcaagtattt ggaaataatg 180
tgtcaattttt gcataatgtg acattgggag ggactggtaa ggcaagtggg gatagacacc 240
ctaagattgg tggatgggttgg ttgataaggc cagggacttgc tattttggggg acattttaaga 300
ttgggtatgg agctaagattt ggtgcttgg ctgttgtgtt gaaggaagtgc ccaccaagga 360
ctactgtgtt tggaaaccctt qctaggttgg ttggagggaa ggataaccctt ataaattgg 420
ataagatgcc tagttttacc atggaccata ctcatggtc tgattatgtt atatagaagc 480
taattaattt tctaaatcatgtt ttttagatgtt gtgttaggtt gggaaattgtt ttgggttgagg 540
gggcttgggtt gtttgtcaag agagaatcta agttctccgt ctgacaacacg ggctgccttt 600
aatcatacgtt gtttagatttt taaagaatag tttagatgttag tactttgttgc ttgtaaagggg 660
ccatgtatgac aaccctttgtt gtaaaatttt tgaatatggc tatttcagct ttgttatgggtt 720
ac

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<210> 14  
<211> 157  
<212> PRT  
<213> Glycine max

<400> 14  
Ala Arg Ala His Lys Leu Trp Leu Gln Gly Arg Lys Val Leu Ala Leu  
1 5 10 15

Leu Ile Gln Asn Arg Val Ser Glu Val Phe Ala Val Asp Ile His Pro  
20 25 30

Gly Ala Lys Ile Gly Arg Gly Ile Leu Leu Asp His Ala Thr Gly Leu  
35 40 45

Val Val Gly Glu Thr Ala Val Ile Gly Asn Asn Val Ser Ile Leu His  
50 55 60

Asn	Val	Thr	Leu	Gly	Gly	Thr	Gly	Lys	Ala	Ser	Gly	Asp	Arg	His	Pro
65	.				70						75				80

Lys Ile Gly Asp Gly Val Leu Ile Gly Ala Gly Thr Cys Ile Leu Gly  
85 90 95

Asn Ile Lys Ile Gly Asp Gly Ala Lys Ile Gly Ala Cys Ser Val Val  
100 105 110

Leu Lys Glu Val Pro Pro Arg Thr Thr Ala Val Gly Asn Pro Ala Arg  
115 120 125

Leu Val Gly Gly Lys Asp Asn Pro Ile Lys Leu Asp Lys Met Pro Ser  
 130 135 140

Phe Thr Met Asp His Thr Ser Trp Ser Asp Tyr Val Ile  
 145 150 155

<210> 15  
 <211> 415  
 <212> DNA  
 <213> Triticum aestivum

<400> 15  
 cggcccgagac gccatcgacg cgacgacggc gacgacgacg acgacgatga cggcggtca 60  
 gccccctccgc gcccggccccc agcagcgccg ccacagcccg cggccctcc accccgcccgt 120  
 ggtgccgtcc taccggcccc cggagtcgg caacgacgag tcctgggtct gggtcccaagat 180  
 caaggccgag gcgcgcgcgcg acgcccacgc cgagccggcg ctgcgtctt tctctacgc 240  
 caccgtgtcc tcccaaaaaactt cgctcgacgc ctccctctcc ttccacactcg ccaacaagct 300  
 ctgctctcc accccctctt ccacgtctt ctacgacctc ttctgggtctt cctcgccgc 360  
 gcaccccaacc atccggccgc cggccgtcgc cgaccccttc gccgtgcgtt cccgg 415

<210> 16  
 <211> 123  
 <212> PRT  
 <213> Triticum aestivum

<400> 16  
 Met Thr Ala Gly Gln Pro Leu Arg Ala Asp Pro Gln Gln Arg Arg His  
 1 5 10 15

Ser Pro Pro Ala Leu His Pro Ala Val Val Pro Ser Tyr Pro Pro Pro  
 20 25 30

Glu Ser Gly Asn Asp Glu Ser Trp Val Trp Ser Gln Ile Lys Ala Glu  
 35 40 45

Ala Arg Arg Asp Ala Asp Ala Glu Pro Ala Leu Ala Ser Phe Leu Tyr  
 50 55 60

Ala Thr Val Leu Ser His Pro Ser Leu Glu Arg Ser Leu Ser Phe His  
 65 70 75 80

Leu Ala Asn Lys Leu Cys Ser Ser Thr Leu Leu Ser Thr Leu Leu Tyr  
 85 90 95

Asp Leu Phe Val Gly Ser Leu Ala Ala His Pro Thr Ile Arg Ala Ala  
 100 105 110

Ala Val Ala Asp Leu Leu Ala Val Arg Ser Arg  
 115 120

<210> 17  
 <211> 294  
 <212> PRT  
 <213> Citrullus lanatus

<400> 17  
 Met Pro Val Gly Glu Leu Arg Phe Ser Ser Gln Ser Ser Thr Thr Val  
 1 5 10 15

Val Glu Ser Thr Thr Asn Asn Asp Glu Thr Trp Leu Trp Gln Gin Ile  
 21 25 31

Lys Ala Glu Ala Arg Arg Asp Ala Glu Ser Glu Pro Ala Leu Ala Ser  
 35 40 45

Tyr Leu Tyr Ser Thr Ile Leu Ser His Ser Ser Leu Glu Arg Ser Leu  
 50 55 60

Ser Phe His Leu Gly Asn Lys Leu Cys Ser Ser Thr Leu Leu Ser Thr  
 65 70 75 80

Leu Leu Tyr Asp Leu Phe Leu Asn Ala Phe Ser Thr Asp Tyr Cys Leu  
 85 90 95

Arg Ser Ala Val Val Ala Asp Leu Gln Ala Ala Arg Glu Arg Asp Pro  
 100 105 110

Ala Cys Val Ser Phe Ser His Cys Leu Leu Asn Tyr Lys Gly Phe Leu  
 115 120 125

Ala Cys Gln Ala His Arg Val Ala His Lys Leu Trp Asn Gln Ser Arg  
 130 135 140

Arg Pro Leu Ala Leu Ala Leu Gln Ser Arg Ile Ala Asp Val Phe Ala  
 145 150 155 160

Val Asp Ile His Pro Ala Ala Arg Ile Gly Lys Gly Ile Leu Phe Asp  
 165 170 175

His Ala Thr Gly Val Val Val Gly Glu Thr Ala Val Ile Gly Asn Asn  
 180 185 190

Val Ser Ile Leu His His Val Thr Leu Gly Gly Thr Gly Lys Met Cys  
 195 200 205

Gly Asp Arg His Pro Lys Ile Gly Asp Gly Val Leu Ile Gly Ala Gly  
 210 215 220

Ala Thr Ile Leu Gly Asn Val Lys Ile Gly Glu Gly Ala Lys Ile Gly  
 225 230 235 240

Ala Gly Ser Val Val Leu Ile Asp Val Pro Pro Arg Thr Thr Ala Val  
 245 250 255

Gly Asn Pro Ala Arg Leu Val Gly Gly Lys Glu Lys Pro Ser Gln Leu  
 260 265 270

Glu Asp Ile Pro Gly Glu Ser Met Asp His Thr Ser Phe Ile Ser Glu  
 275 280 285

Trp Ser Asp Tyr Ile Ile  
 290

<210> 18  
 <211> 312  
 <212> PRT  
 <213> *Arabidopsis thaliana*

<400> 18  
Met Pro Pro Ala Gly Glu Leu Arg His Gln Ser Pro Ser Lys Glu Lys  
1 5 10 15  
Leu Ser Ser Val Thr Gln Ser Asp Glu Ala Glu Ala Ala Ser Ala Ala  
20 25 30  
Ile Ser Ala Ala Ala Asp Ala Glu Ala Ala Gly Leu Trp Thr Gln  
35 40 45  
Ile Lys Ala Glu Ala Arg Arg Asp Ala Glu Ala Glu Pro Ala Leu Ala  
50 55 60  
Ser Tyr Leu Tyr Ser Thr Ile Leu Ser His Ser Ser Leu Glu Arg Ser  
65 70 75 80  
Ile Ser Phe His Leu Gly Asn Lys Leu Cys Ser Ser Thr Leu Leu Ser  
85 90 95  
Thr Leu Leu Tyr Asp Leu Phe Leu Asn Thr Phe Ser Ser Asp Pro Ser  
100 105 110  
Leu Arg Asn Ala Thr Val Ala Asp Leu Arg Ala Ala Arg Val Arg Asp  
115 120 125  
Pro Ala Cys Ile Ser Phe Ser His Cys Leu Leu Asn Tyr Lys Gly Phe  
130 135 140  
Leu Ala Ile Gln Ala His Arg Val Ser His Lys Leu Trp Thr Gln Ser  
145 150 155 160  
Arg Lys Pro Leu Ala Leu Ala Leu His Ser Arg Ile Ser Asp Val Phe  
165 170 175  
Ala Val Asp Ile His Pro Ala Ala Lys Ile Gly Lys Gly Ile Leu Leu  
180 185 190  
Asp His Ala Thr Gly Val Val Gly Glu Thr Ala Val Ile Gly Asn  
195 200 205  
Asn Val Ser Ile Leu His His Val Thr Leu Gly Gly Thr Gly Lys Ala  
210 215 220  
Cys Gly Asp Arg His Pro Lys Ile Gly Asp Gly Cys Leu Ile Gly Ala  
225 230 235 240  
Gly Ala Thr Ile Leu Gly Asn Val Lys Ile Gly Ala Gly Ala Lys Val  
245 250 255  
Gly Ala Gly Ser Val Val Leu Ile Asp Val Pro Cys Arg Gly Thr Ala  
260 265 270  
Val Gly Asn Pro Ala Arg Leu Val Gly Gly Lys Glu Lys Pro Thr Ile  
275 280 285  
His Asp Glu Glu Cys Pro Gly Glu Ser Met Asp His Thr Ser Phe Ile  
290 295 300  
Ser Glu Trp Ser Asp Tyr Ile Ile  
305 310

<210> 19  
 <211> 336  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 19  
 Met Ala Ala Cys Ile Asp Thr Cys Arg Thr Gly Lys Pro Gln Ile Ser  
 1 5 10 15

Pro Arg Asp Ser Ser Lys His His Asp Asp Glu Ser Gly Phe Arg Tyr  
 20 25 30

Met Asn Tyr Phe Arg Tyr Pro Asp Arg Ser Ser Phe Asn Gln Thr Gln  
 35 40 45

Thr Lys Thr Leu His Thr Arg Pro Leu Leu Glu Asp Leu Asp Arg Asp  
 50 55 60

Ala Glu Val Asp Asp Val Trp Ala Lys Ile Arg Glu Glu Ala Lys Ser  
 65 70 75 80

Asp Ile Ala Lys Glu Pro Ile Val Ser Ala Tyr Tyr His Ala Ser Ile  
 85 90 95

Val Ser Gln Arg Ser Leu Glu Ala Ala Leu Ala Asn Thr Leu Ser Val  
 100 105 110

Lys Leu Ser Asn Leu Asn Leu Pro Ser Asn Thr Leu Phe Asp Leu Phe  
 115 120 125

Ser Gly Val Leu Gln Gly Asn Pro Asp Ile Val Glu Ser Val Lys Leu  
 130 135 140

Asp Leu Leu Ala Val Lys Glu Arg Asp Pro Ala Cys Ile Ser Tyr Val  
 145 150 155 160

His Cys Phe Leu His Phe Lys Gly Phe Leu Ala Cys Gln Ala His Arg  
 165 170 175

Ile Ala His Glu Leu Trp Thr Gln Asp Arg Lys Ile Leu Ala Leu Leu  
 180 185 190

Ile Gln Asn Arg Val Ser Glu Ala Phe Ala Val Asp Phe His Pro Gly  
 195 200 205

Ala Lys Ile Gly Thr Gly Ile Leu Leu Asp His Ala Thr Ala Ile Val  
 210 215 220

Ile Gly Glu Thr Ala Val Val Gly Asn Asn Val Ser Ile Leu His Asn  
 225 230 235 240

Val Thr Leu Gly Gly Thr Gly Lys Gln Cys Gly Asp Arg His Pro Lys  
 245 250 255

Ile Gly Asp Gly Val Leu Ile Gly Ala Gly Thr Cys Ile Leu Gly Asn  
 260 265 270

Ile Thr Ile Gly Glu Gly Ala Lys Ile Gly Ala Gly Ser Val Val Leu  
 275 280 285

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Lys Asp Val Pro Pro Arg Thr Thr Ala Val Gly Asn Pro Ala Arg Leu  
290 295 300  
Leu Gly Gly Lys Asp Asn Pro Lys Thr His Asp Lys Ile Pro Gly Leu  
305 310 315 320  
Thr Met Asp Gln Thr Ser His Ile Ser Glu Trp Ser Asp Tyr Val Ile  
325 330 335